

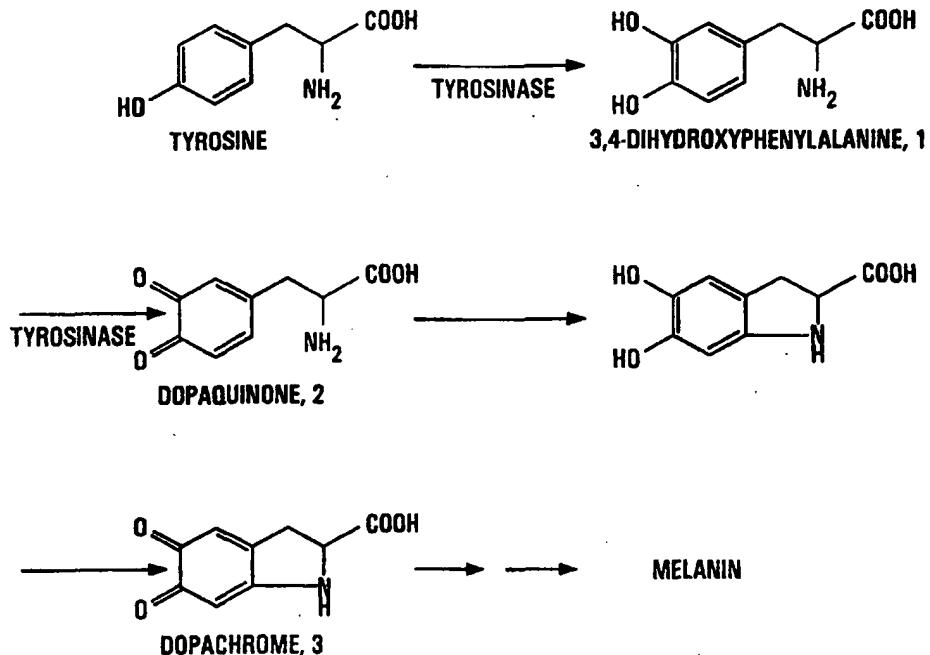


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## (54) Title: TYROSINASE INHIBITORS FROM PLANTS



## (57) Abstract

The present invention provides tyrosinase inhibiting extracts from dicotyledonous plant species indigenous to Canada and compositions containing the extracts for the treatment of skin, particularly skin lightening. The present invention also provides a method of detecting tyrosinase activity in an extract of a dicotyledonous plant species indigenous to Canada.

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## Tyrosinase Inhibitors from Plants

Field of the Invention

The invention relates to a product comprising a tyrosinase inhibiting extract derived from a dicotyledonous plant species 5 indigenous to Canada. The present invention also relates to compositions comprising the product and a suitable diluent or carrier for the treatment of skin or of fruit, vegetables or beverages. The present invention further relates to a method for identifying plant extracts that are capable of inhibiting 10 the enzyme tyrosinase.

Background of the Invention

Tyrosinase (tyrosine oxidase, EC 1.10.3.1) is an enzyme or closely homologous group of enzymes of universal occurrence in microbes, plants and animals. Its primary metabolic function 15 is to catalyze the oxidative degradation of the amino acid tyrosine. This degradation takes slightly different routes in animals, plants and microbes, but the rate-controlling first steps - those catalyzed by tyrosinase - are the same in virtually all living species. In animals, including man, 20 tyrosinase first transforms tyrosine into 3,4-dihydroxyphenylalanine (DOPA), thence to the corresponding quinone (DOPAquinone), and finally to 2-carboxy-2,3-dihydroindole-5,6-quinone (DOPAchrome) which is further converted by other enzymes to still more highly oxidized 25 materials which include the melanin substances responsible for skin pigmentation.

Skin pigmentation thus depends upon the action of tyrosinase. If this enzyme is not active, normal pigmentation does not occur, and skin loses or fails to acquire its normal 30 tan-to-brown coloration. The effect is independent of racial or environmental factors. Pigmentation loss manifests itself in *lentigo senile*, so-called "age spots", a small-scale, patchy color loss often seen in skin of people over 50 years of age; typically the de-pigmented patches will be 1-3 mm across and 35 can be very numerous. A more serious effect is *leucoderma*, a group of diseases in which large areas of skin lose their melanin and appear pink. An extreme effect is *albinism*, a

condition in which tyrosinase enzyme is entirely dysfunctional and no pigmentation of skin (or eyes or hair) occurs.

If on the other hand tyrosinase activity is accelerated, as in sun-tanning or in some pathological conditions, the amount of melanin formed increases and skin color darkens. When the distribution of the new melanin is even and controlled, the result is a "glorious bronzed body"; but if the new melanin is patchy or produced uncontrollably, the result is pathological. Skin *melanomas* are sites of localized hyper-activity by tyrosinase; they are often associated with cancerous cell modification. The causal relationship of tyrosinase action to skin pigmentation was established many years ago (Mason, H. S. (1948) *J. Biol. Chem.* 172, 83-86; Balin, A. K. and Kligman, A. M. (1989) *Aging and the Skin* 372pp, Raven Press, New York) and is now well-understood.

Materials which suppress the action of tyrosine oxidase are presumed to slow the build-up of skin spots over time, and this presumption is vindicated by scientific evidence. Thus, the correlation between tyrosinase inhibition and protection of skin against unwanted pigmentation is accepted by the medical profession and by the cosmetic industry.

In addition to its skin functions, tyrosinase is active in other types of living tissue in the turnover metabolism of tyrosine and in the production of pigmented materials in those tissues.

With respect to cosmetics, skin lightening and skin darkening have been practiced since earliest times, and today these are mainstays of some sectors of the world cosmetics industry. Skin lightening has traditionally been accomplished by rigorously excluding sunlight from skin, or by the use of chemical lightening agents. One such agent is synthetic hydroquinone (1,4-dihydroxybenzene). This substance is considered the cosmetic industry standard for tyrosinase inhibition potency.

Summary of the Invention

Prior to the present invention, the possibility of finding effective tyrosinase inhibitors in dicotyledonous plant species indigenous to Canada had not been appreciated.

5        Accordingly, the present invention provides a product comprising an extract derived from a dicotyledonous plant species indigenous to Canada, wherein the extract inhibits tyrosinase. Preferably, the extract is derived from a plant species selected from Polygonaceae, Rosaceae and Onagraceae.

10      The extract is preferably derived from one or more parts of the plant selected from leaves, twigs, flowers, flowering aerials, fruiting aerials, seeding aerials, roots and fruits.

      Preferably, the extract is derived from the group consisting of:

15      flowering aerials of *Artemisia campestris* (plains wormwood);  
              flowering aerials of *Aster ericoides* (white prairie aster);  
              flowering aerials of *Aster hesperius* (willow aster);  
              leaves, twigs and flowers of *Cornus stolonifera* (red-osier dogwood);

20      leaves and twigs of *Cotoneaster acutifolia* (cotoneaster);  
              flowering aerials of *Epilobium angustifolium* (fireweed);  
              seedling aerials of *Euphorbia esula* (leafy spurge);  
              fruiting aerials of *Fragaria americana* (wild strawberry);  
              fruiting aerials of *Fragaria glauca* (Wild strawberry);

25      flowering aerials of *Geranium bicknelli* (Bicknell's geranium);  
              flowering aerials of *Geum aleppicum* (yellow avens);  
              flowering aerials of *Geum triflorum* (3-flowered avens);  
              flowering aerials of *Glycyrrhiza lepidota* (wild licorice);  
              flowering aerials of *Hedysarum americanum* (American hedysarum);

30      roots of *Heuchera richardsonii* (alumroot);  
              flowering aerials of *Oenothera biennis* (yellow evening-primrose);  
              flowering aerials of *Polygonum persicaria* (lady's-thumb);  
              flowering aerials of *Potentilla fruticosa* (shrubby cinquefoil);

35      flowering aerials of *Potentilla norvegica* (rough cinquefoil);  
              flowering aerials of *Rosa acicularis* (prairie rose);  
              fruiting aerials of *Rosa arkansana* (low rose);

fruiting aerials of *Rumex maritimus* (golden dock);  
fruiting aerials and flowering aerials of *Rumex occidentalis* (western field dock);  
fruiting aerials and fruits of *Rumex pseudonatronatus* (field dock); and  
5 fruits of *Rumex stenophyllus* (narrow-leaved dock).

The present invention also provides a composition comprising the product as defined herein, together with a 10 cosmetically or pharmaceutically acceptable, or edible, diluent or carrier. The composition is preferably for cosmetic treatment of skin or for inhibiting browning of edible products. The composition is more preferably for skin lightening, even more preferably to reduce melanin and/or 15 melanogenesis. The at least one plant extract in the composition is preferably selected from the group consisting of:

fruiting aerials of *Rumex maritimus* (golden dock);  
fruiting aerials of *Rumex occidentalis* (western field dock);  
flowering aerials of *Rumex occidentalis* (western field dock);  
20 fruiting aerials of *Rumex pseudonatronatus* (field dock);  
fruits of *Rumex pseudonatronatus* (field dock); and  
fruits of *Rumex stenophyllus* (narrow-leaved dock).

The present invention also provides a method for detecting 25 tyrosinase inhibiting activity in an extract derived from a dicotyledonous plant species indigenous to Canada. This method comprises:

- a) preparing a first solution comprising an amount of tyrosinase and a suitable substrate;
- b) preparing a second solution comprising the same 30 amount of tyrosinase and the suitable substrate and further comprising an amount of the extract;
- c) measuring tyrosinase activities of the first and second solutions by suitable methods;
- d) comparing the tyrosinase activities of the first and 35 second solutions; and
- e) detecting tyrosinase inhibiting activity, present when the tyrosinase activity of the second solution is less

than the tyrosinase activity of the first solution.

It is preferable that the tyrosinase inhibiting activity is comparable to, or greater than, that of hydroquinone.

Brief Description of the Drawings

5 Figure 1 is summary of the main steps in the biogenesis of melanin from tyrosine.

Figure 2 is a plot of "percent inhibition of enzyme action" vs. log "percent extract in assay reaction mixture" indicating the effectiveness of extracts of Rumex

10 pseudonatronatus, Rumex occidentalis and Oenothera biennis as tyrosinase inhibitors. Tyrosinase-5mg. Standard-hydroquinone.

Figure 3 is a plot of "percent inhibition of enzyme action" vs. log "percent extract in assay reaction mixture" indicating the effectiveness of extracts of Hedysarum

15 americanum and Rosa acicularis as tyrosinase inhibitors.

Tyrosinase - 5mg. Standard - hydroquinone.

Figure 4 shows the elution profile of whole extract of Rumex pseudonatronatus from a polyvinylpyrrolidone chromatographic column in terms of percent inhibition of 20 tyrosinase in the assay. Elution was with water (ff 1-4) and water-ethanol gradient (ff 5-10).

Figure 5 shows the effect of alcohol in the extraction solvent on tyrosinase inhibiting activity from Epilobium angustifolium. Epilobium angustifolium (=WH) was 25 extracted using water (WH-00), 25% ethanol (WH-25), 50% ethanol (WH-50), 75% ethanol (WH-75) and 95% ethanol (WH-95). Each extract was assayed for tyrosinase inhibitory power at three concentrations. The carrier was butanediol and water.

Description of the Preferred Embodiments

30 The definitions of various terms used in this specification appear below.

"Plant extracts" are substances obtained from plants by physical separation processes such as solvent extraction, steam distillation, or pressing. Broadly, the term "extract" may 35 sometimes be extended to include the solvent as well as the plant substance dissolved in it.

The term "screening test" refers to any simple and

standardized test whose purpose is to separate or screen out, from a large number of materials, those unable to perform the function upon which the test is based, from those able to perform that function.

5 A typical pilot scale plant extraction method for Rumex species is provided below.

Ripe, dry (post-season) Rumex species, mostly *R. Occidentalis*, were field collected and dry-milled into small fragments. These (250kg) were extracted at 70°C for one hour 10 with 2000 L of approximately 70% ethanol in water. An orange extract was separated from the mash by decantation and the latter was washed with a further 1000 L of 70% alcohol. The extract liquids were combined, filtered and concentrated to 50 L, by which point the ethanol had virtually all been removed 15 and extract solids (content 18-20%) were then beginning to precipitate out. Alternatively, 50 L of glycerol or 1,3-butanediol was added to the partly concentrated solution to provide a final concentration end-point volume of 100 L. This approach avoids precipitation of extract solids.

20 Carrier glycerol (or 1,3-butanediol) and water were added to the concentrate to make a red-coloured solution having 5% solids content. Then a further amount of 1:1 glycerol (or 1,3-butanediol)-water carrier liquid was added, amounting to 25% of the weight of the 5% solution and itself containing 5% of L- 25 ascorbic acid (Vitamin C) as an anti-colorant and anti-oxidant. Finally, an anti-microbial preservative, such as phenoxyethanol, was added to a concentration of 0.3 to 0.4%.

The above method provides the basis for the method used to produce the other extracts of the invention. A person skilled 30 in the art will understand that variation in certain parameters and substances used in the method will still produce an extract of the invention.

The tyrosinase assay was based on methods previously published (Mason, H.S., (1948) *J. Biol. Chem.* 172, 83-86; 35 Dawson, C.R. and Magee, R.J. (1955) *Methods in Enzymology* II 817-827 (Editors-in-chief S.P. Colowick and N.O. Caplan); Lerner, A.B. (1955) *ibid* 827-831; Bergmeyer, U.H. (1983)

Methods of Enzymatic Analysis (Published by Verlag Chemie, Weinheim)). The protocol was as follows, scaled for the approximately twenty sample determinations:

(a) A buffer solution of pH 6.5 was prepared by 5 dissolving 1.4 g disodium monohydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) and 1.4 g monopotassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) in pure water to make 200 mL.

(b) An enzyme solution of either 1.0 mg or 3.0 mg 10 tyrosinase (Sigma T-7755) was prepared in 30 mL buffer and stored at 2°C until use.

(c) A substrate solution was prepared by dissolving 0.10g L-DOPA (L-3,4-dihydroxyphenyl-alanine, Sigma D-9661) in 100 mL buffer.

(d) The unfettered enzyme reaction rate was measured by 15 mixing together 0.5 mL buffer solution and 0.5 mL substrate solution in twin acrylate mini-cuvettes (path 2.0 mm), and after observing the stability of the mixture, mixing in 0.25 mL enzyme solution to one cuvette. The formation of red DOPAquinone was monitored spectrophotometrically at 475 nm, at 20 times 0, 1, 2 and 3 minutes.

(e) The effect of added substances on the enzyme reaction rate was measured by mixing together 0.5 mL buffer solution, 0.5 mL substrate solution and 0.25 mL test substance (at 1% strength in 50:50 butyleneglycol:water) in twin acrylate mini- 25 cuvette. The reaction was tracked spectrophotometrically at 475 nm, reading at 0, 1, 2 and 3 minutes.

(f) The effect of test substances on reaction rate was expressed as inhibition (I) where  $I = 100 - [100 \text{ (trt absorbance change, 3min)} / 100 \text{ (control absorbance change, 3min)}]$ . 30

Concentrations of substrate, enzyme or test substance can be varied as appropriate.

Plant extracts screened by the method of the invention were selected which exhibited tyrosinase inhibiting potency 35 equal to, or better than, hydroquinone. Those extracts are listed in Table 1. The concentration dependence of the potency of various extracts is summarized in Figures 2 and 3 in

comparison to the concentration dependence of the potency of hydroquinone.

Table 2 shows the effect of varying the extraction solvent on the tyrosinase inhibiting activity of extracts from three 5 different plant species. The effect is also shown by the results in Figure 5.

TABLE 1: Plant extracts and phytochemicals with tyrosinastatic power equal to or greater than that of hydroquinone

<u>Plant botanical name</u>	<u>Common name</u>	<u>Parts used</u>	<u>Enzymes reaction rate reduction</u>	
			<u>@ 5 <math>\mu</math>g enzyme</u>	<u>@ 15 <math>\mu</math>g enzyme</u>
			<u>65%</u>	<u>55%</u>
<b>PLANT EXTRACTS OF</b>				
Amalanchier alnifolia	Saskatoonberry	flwrq, leaves & twigs	83%, 85%	nd
Artemisia campestris	plains wormwood	flowering aerials	nd	65%
Aster ericoides	white prairie aster	flowering aerials	nd	78%
Aster hesperius	willow aster	flowering aerials	66%	nd
Cornus stolonifera	red-osier dogwood	leaves, twigs, flowers	79%	60%
Cotoneaster acutifolia	cotoneaster	leaves & twigs	65%	nd
Epilobium angustifolium A	firewood	flowering aerials	70%	61%
Epilobium angustifolium B	firewood	flowering aerials	82%	nd
Euphorbia esula	leafy spurge	seedling aerials	nd	58%
Fragaria americana	wild strawberry	fruiting aerials	nd	61%
Fragaria glauca	wild strawberry	fruiting aerials	77%, 68%	72%
Geranium bicknellii	Bicknell's geranium	flowering aerials	64%	61%
Geum aleppicum	Yellow avens	flowering aerials	78%	67%
Geum triflorum	3-flowered avens	flowering aerials	78%	71%
Glycyrrhiza lepidota A	wild licorice	flowering aerials	76%	nd
Hedysarum americanum B	American hedysarum	flowering aerials	93%, 87%, 90%	88%

TABLE 1 (cont.)

<u>Plant botanical name</u>	<u>Common name</u>	<u>Parts used</u>	<u>Enzymes reaction rate reduction</u>
Standard: pure HYDROQUINONE @ 1%			<u>@ 5 <math>\mu</math>g enzyme</u> <u>65%</u>
PLANT EXTRACTS OF			<u>@ 15 <math>\mu</math>g enzyme</u> <u>55%</u>
<i>Heuchera richardsonii</i>	alumroot	roots	90%
<i>Oenothera biennis</i>	yellow evening-primrose	flowering aerials	82%
<i>Polygonum persicaria</i>	lady's-thumb	flowering aerials	90%
<i>Potentilla fruticosa</i>	shrubby cinquefoil	flowering aerials	83%, 78%
<i>Potentilla norvegica</i>	rough cinquefoil	flowering aerials	78%
<i>Rosa acicularis</i>	prairie rose	flowering aerials	83%
<i>Rosa arkansana</i>	low rose	fruiting aerials	81%
<i>Rumex maritimus</i>	golden dock	fruiting aerials	90%
<i>Rumex occidentalis A</i>	western field dock	fruiting aerials	87%
<i>Rumex occidentalis B</i>	western field dock	flowering aerials	nd
<i>Rumex pseudonatronatus A</i>	field dock	fruiting aerials	92%
<i>Rumex pseudonatronatus B</i>	field dock	fruits	88%
<i>Rumex stenophyllus</i>	narrow-leaved dock	fruits	88%
			69%

TABLE 2: Extractability of tyrosinastatic activity from plants

The grid numbers are the percent inhibitions of the enzyme function obtained using each extracted solution in two standard tyrosinase assays (5 µg enzyme). Data are corrected for ethanol effects.

SPECIES	EXTRACTION	SOLVENT	
<i>Epilobium angustifolium</i> (aerials)	Ethanol	1:1 Water-ethanol	Ethyl acetate
<i>Oenothera biennis</i> (aerials)		65%, 62%	<10%
<i>Rumex occidentalis</i> (ripe aerials)		80%, 75%	<101%
		58%, 67%	66%, 63%
		79%, 79%	81%, 84%
			<10%

There is a very large market for consumer goods containing natural products, such as plant extracts. Compositions for treatment of skin may be prepared comprising a tyrosinase inhibiting plant extract as disclosed herein, together with an acceptable diluent or carrier. Acceptable diluent or carriers for cosmetic applications can include such substances as water, aqueous alcohols, glycerol, propanediol and butanediol, as well as certain silicones and waxes. Acceptable diluents or carriers for pharmaceutical applications are well known in the art.

The tyrosinase inhibiting plant extract can also be used to treat fruit, vegetables or beverages to prevent or reduce browning, since tyrosinase activity is a contributor to the browning process. Compositions may be prepared to treat edible products such as fruit, vegetables or beverages to inhibit browning, which composition comprises a tyrosinase inhibiting plant extract as disclosed herein, together with a diluent or carrier that is suitable for consumption. The diluent or carrier suitable for consumption can include such substances as water, aqueous alcohols, propanediol and butanediol, as well as any other suitable substance acceptable by law.

**EXAMPLE 1**

A composition known as TYROSTAT-10 (T-10) has the following ingredients:

30 L-Ascorbic acid 0.9-1.1%  
Phenoxyethanol (anti-fungal) 0.2-0.3%  
The composition contains no added minerals or other  
organic materials. It is a readily pourable transparent  
solution, orange-red coloured at pH 4.0-7.5. Upon dilution,  
35 the colour moves toward pastel yellow. This colour darkens  
perceptibly but reversibly at pH values much above 8, and  
lightens perceptibly on long product storage when sealed from

air.

The specific gravity is 1.05 at 21°C.

This material contains natural plant-sourced inhibitors of tyrosinase enzymes. The potency has been measured using *in vitro* assays of the conversion of DOPA (3,4-dihydroxyphenylalanine) to the corresponding quinone by the enzyme, in presence and in absence of the TYROSTAT product and the results are summarized in Table 3. This assay duplicates rate-controlling steps in the human metabolic pathway to skin pigmentation. Pure hydroquinone in 1% solution was employed as a comparison standard.

**TABLE 3**

<u>TESTED MATERIAL</u>	<u>SOLIDS PRESENT</u>	<u>INHIBITION OF ENZYME</u>
Undiluted TYROSTAT-10	5%	100%
TYROSTAT-10 diluted 10X	0.5	100%
TYROSTAT-10 diluted 100X	0.05	80-85%
Hydroquinone	1.0	58-62%

Rumex extracts appear to inhibit tyrosinase by binding or deforming the enzyme molecule around the active site. Ascorbic acid contributes to the overall inhibition by maintaining the enzyme's copper atoms in their reduced Cu<sup>+</sup> state. Other constituents of TYROSTAT-10 have no significant effect on tyrosinase in standard assays, although the carrier component butanediol is slightly inhibitory. The data in Table 4 indicate the relative capabilities of the active ingredients for tyrosinase inhibition, at various concentrations of the ingredients.

**TABLE 4**

30	Ingredient	Inhibition of tyrosinase enzyme step 2			
		1% conc	0.1% conc	0.01% conc	0.001% conc
	Rumex extract	81%	69%	53%	38%
	Ascorbic acid	100%	100%	82%	23%
	[cf. hydroquinone]	60%	31%	<10%	<10%

EXAMPLE 2

A composition known as TYROSTAT-11 (T-11) has the following ingredients:

	Purified Water	45-50%
5	Glycerol	45-50%
	Plant extract solids (Canadian Rumex species) [Rumex occidentalis, R. pseudonatronatus, R. stenophyllus and R. maritimus]	4.0-4.3%
10	L-Ascorbic acid	0.9-1.1%
	Phenoxyethanol (anti-fungal)	0.2-0.3%

The composition contains no added minerals or other inorganic materials, and no synthetics other than phenoxyethanol preservative. It is a readily pourable 15 transparent solution, orange-red coloured at pH 4.0-7.5. Upon dilution, the colour moves toward pastel yellow. This colour darkens perceptibly but reversibly at pH values much above 8, and lightens perceptibly on long product storage when sealed from air.

20 The specific gravity is 1.15 at 21°C.

This material contains natural plant-sourced inhibitors of tyrosinase enzymes. The potency has been measured using *in vitro* assays of the conversion of DOPA (3,4-dihydroxyphenylalanine) to the corresponding quinone by the 25 enzyme, in the presence and in absence of the TYROSTAT product and the results are summarized in Table 5. This assay duplicates rate-controlling steps in the human metabolic pathway to skin pigmentation. Pure hydroquinone in 1% solution was employed as a comparison standard.

30 TABLE 5

<u>TESTED MATERIAL</u>	<u>SOLIDS PRESENT</u>	<u>INHIBITION OF ENZYME</u>
Undiluted TYROSTAT-11	5%	100%
TYROSTAT-11 diluted 10X	0.5	100%
TYROSTAT-11 diluted 100X	0.05	80-83%
35 Hydroquinone	1.0	38-62%

Rumex extracts appear to inhibit tyrosinase by binding or deforming the enzyme molecule around the active site. Ascorbic

acid contributes to the overall inhibition by maintaining the enzyme's copper atoms in their reduced Cu<sup>+</sup> state. Other constituents of TYROSTAT-11 have no significant effect on tyrosinase in standard assays. The data in Table 6 indicate 5 the relative capabilities of the active ingredients for tyrosinase inhibition, at various concentrations of those ingredients.

**TABLE 6**

10	Ingredient	Inhibition of tyrosinase enzyme step 2			
		1% conc	0.1% conc	0.01% conc	0.001% conc
	Rumex extract	81%	69%	53%	38%
	Ascorbic acid	100%	100%	82%	23%
	[cf. hydroquinone]	60%	31%	<10%	<10%

15 Numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practised otherwise than as specifically described herein.

## Claims:

1. A product comprising an extract derived from a dicotyledonous plant species indigenous to Canada, wherein the 5 extract inhibits tyrosinase.
2. The product according to claim 1, wherein the extract is derived from a plant species selected from Polygonaceae, Rosaceae and Onagraceae.  
10
3. The product according to claim 1, wherein the extract is derived from one or more parts of the plant selected from leaves, twigs, flowers, flowering aerials, fruiting aerials, seeding aerials, roots and fruits.  
15
4. The product according to claim 1, wherein the extract is derived from the group consisting of:  
flowering aerials of Artemisia campestris (plains wormwood);  
flowering aerials of Aster ericoides (white prairie aster);  
20 flowering aerials of Aster hesperius (willow aster);  
leaves, twigs or flowers of Cornus stolonifera (red-osier dogwood);  
leaves and twigs of Cotoneaster acutifolia (cotoneaster);  
flowering aerials of Epilobium angustifolium (fireweed);  
25 seeding aerials of Euphorbia esula (leafy spurge);  
fruiting aerials of Fragaria americana (wild strawberry);  
fruiting aerials of Fragaria glauca (Wild strawberry);  
flowering aerials of Geranium bicknellii (Bicknell's geranium);  
flowering aerials of Geum aleppicum (yellow avens);  
30 flowering aerials of Geum triflorum (3-flowered avens);  
flowering aerials of Glycyrrhiza lepidota (wild licorice);  
flowering aerials of Hedysarum americanum (American hedysarum);  
roots of Heuchera richardsonii (alumroot);  
flowering aerials of Oenothera biennis (yellow  
35 evening-primrose); flowering aerials of Polygonum persicaria (lady's-thumb);  
flowering aerials of Potentilla fruticosa (shrubby cinquefoil);

flowering aerials of *Potentilla norvegica* (rough cinquefoil);  
flowering aerials of *Rosa acicularis* (prairie rose);  
fruiting aerials of *Rosa arkansana* (low rose);  
fruiting aerials of *Rumex maritimus* (golden dock);  
5 fruiting aerials and flowering aerials of *Rumex occidentalis* (western field dock);  
fruiting aerials and fruits of *Rumex pseudonatronatus* (field dock); and  
fruits of *Rumex stenophyllus* (narrow-leaved dock).

10 5. A composition comprising the product as defined in claim 1, together with a cosmetically or pharmaceutically acceptable, or edible, diluent or carrier.

15 6. A composition comprising the product as defined in claim 4, together with a cosmetically or pharmaceutically acceptable, or edible, diluent or carrier.

20 7. The composition according to claim 5, wherein the diluent or carrier is cosmetically acceptable and the composition is for cosmetic treatment of skin.

25 8. The composition according to claim 7, wherein the extract is derived from the group consisting of:  
fruiting aerials of *Rumex maritimus* (golden dock);  
fruiting aerials of *Rumex occidentalis* (western field dock);  
flowering aerials of *Rumex occidentalis* (western field dock);  
fruiting aerials of *Rumex pseudonatronatus* (field dock);  
fruits of *Rumex pseudonatronatus* (field dock); and  
30 fruits of *Rumex stenophyllus* (narrow-leaved dock).

35 9. The composition according to claim 8, which further comprises ascorbic acid.

10. Use of the composition according to claim 7 as a skin lightener.

11. Use of the composition according to claim 7 to reduce melanin and/or melanogenesis in skin.

12. The composition according to claim 5, wherein the diluent 5 or carrier is edible and the composition is for treating edible products to inhibit browning.

13. A method for detecting tyrosinase inhibiting activity in an extract derived from a dicotyledonous plant species 10 indigenous to Canada, which method comprises:

- a) preparing a first solution comprising an amount of tyrosinase and a suitable substrate;
- b) preparing a second solution comprising the same amount of tyrosinase and the suitable substrate and further 15 comprising an amount of the extract;
- c) measuring a tyrosinase activity of each of the first and second solutions;
- d) comparing the tyrosinase activities of the first and second solutions; and
- 20 e) detecting tyrosinase inhibiting activity, present when the tyrosinase activity of the second solution is less than the tyrosinase activity of the first solution.

14. The method according to claim 13, wherein tyrosinase 25 activity is measured spectrophotometrically.

15. The method according to claim 13, wherein the substrate is L-DOPA.

30 16. The method according to claim 13, wherein the tyrosinase inhibiting activity is equal to, or greater than, that of hydroquinone (1,4-dihydroxybenzene).

35 17. The method according to claim 13, wherein the extract is derived from the group consisting of:  
flowering aerials of *Artemisia campestris* (plains wormwood);

flowering aerials of *Aster ericoides* (white prairie aster);  
flowering aerials of *Aster hesperius* (willow aster);  
leaves, twigs and flowers of *Cornus stolonifera* (red-osier dogwood);

5 leaves or twigs of *Cotoneaster acutifolia* (cotoneaster);  
flowering aerials of *Epilobium angustifolium* (fireweed);  
seedling aerials of *Euphorbia esula* (leafy spurge);  
fruiting aerials of *Fragaria americana* (wild strawberry);  
fruiting aerials of *Fragaria glauca* (Wild strawberry);

10 flowering aerials of *Geranium bicknelli* (Bicknell's geranium);  
flowering aerials of *Geum aleppicum* (yellow avens);  
flowering aerials of *Geum triflorum* (3-flowered avens);  
flowering aerials of *Glycyrrhiza lepidota* (wild licorice);  
flowering aerials of *Hedysarum americanum* (American hedysarum);

15 roots of *Heuchera richardsonii* (alumroot);  
flowering aerials of *Oenothera biennis* (yellow evening-primrose); flowering aerials of *Polygonum persicaria* (lady's-thumb);  
flowering aerials of *Potentilla fruticosa* (shrubby cinquefoil);

20 flowering aerials of *Potentilla norvegica* (rough cinquefoil);  
flowering aerials of *Rosa acicularis* (prairie rose);  
fruiting aerials of *Rosa arkansana* (low rose);  
fruiting aerials of *Rumex maritimus* (golden dock);  
fruiting aerials and flowering aerials of *Rumex occidentalis*

25 (western field dock);  
fruiting aerials and fruits of *Rumex pseudonatronatus* (field dock); and  
fruits of *Rumex stenophyllus* (narrow-leaved dock).

30 18. The method according to claim 17, wherein the extract is derived from the group consisting of:  
fruiting aerials of *Rumex maritimus* (golden dock);  
fruiting aerials of *Rumex occidentalis* (western field dock);  
flowering aerials of *Rumex occidentalis* (western field dock);

35 fruiting aerials of *Rumex pseudonatronatus* (field dock);  
fruits of *Rumex pseudonatronatus* (field dock); and  
fruits of *Rumex stenophyllus* (narrow-leaved dock).

19. A tyrosinase inhibiting plant extract having tyrosinase inhibiting potency at least equal to hydroquinone.

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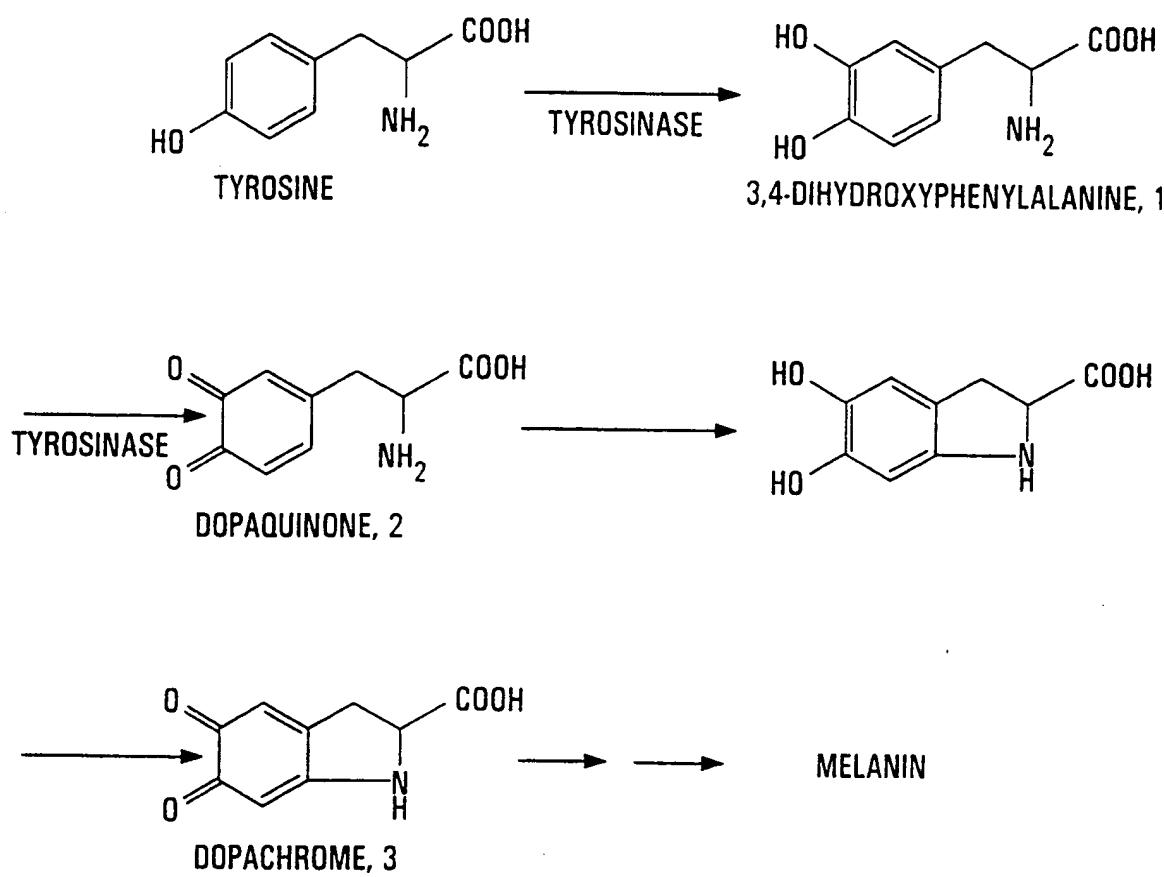


FIG. 1

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## RUMEX OCCIDENTALIS

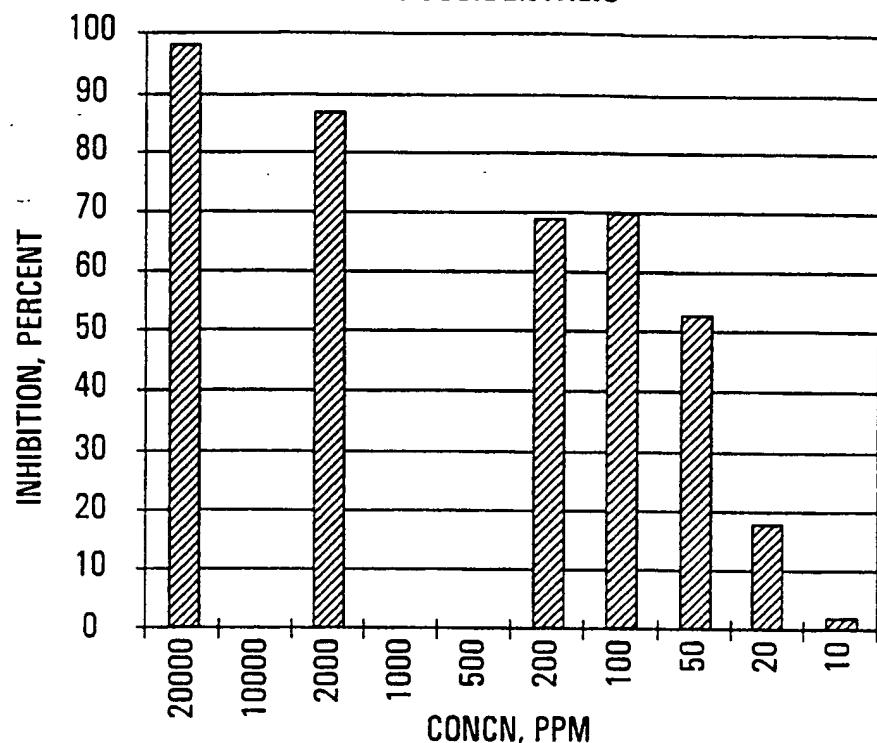


FIG. 2A

## RUMEX PSEUDONATRONATUS AS TYROSTAT

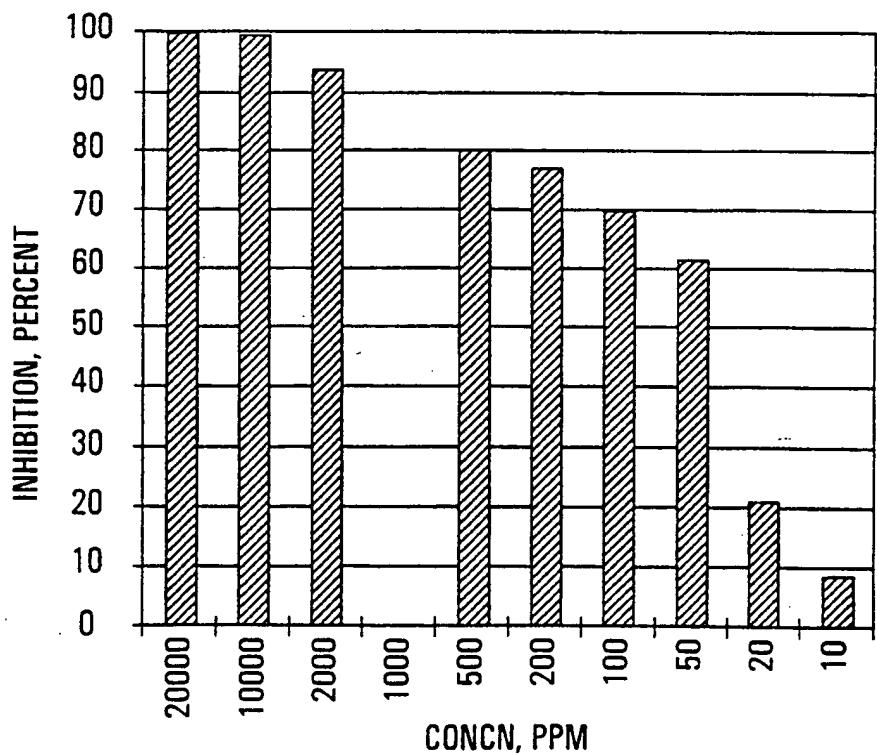


FIG. 2B

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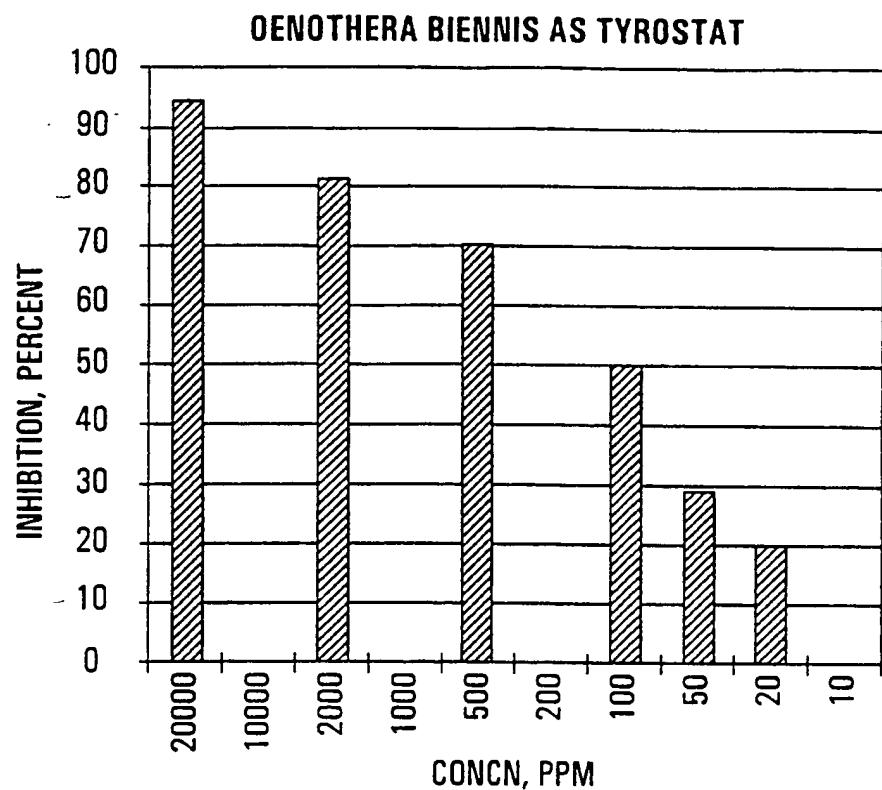


FIG. 2C

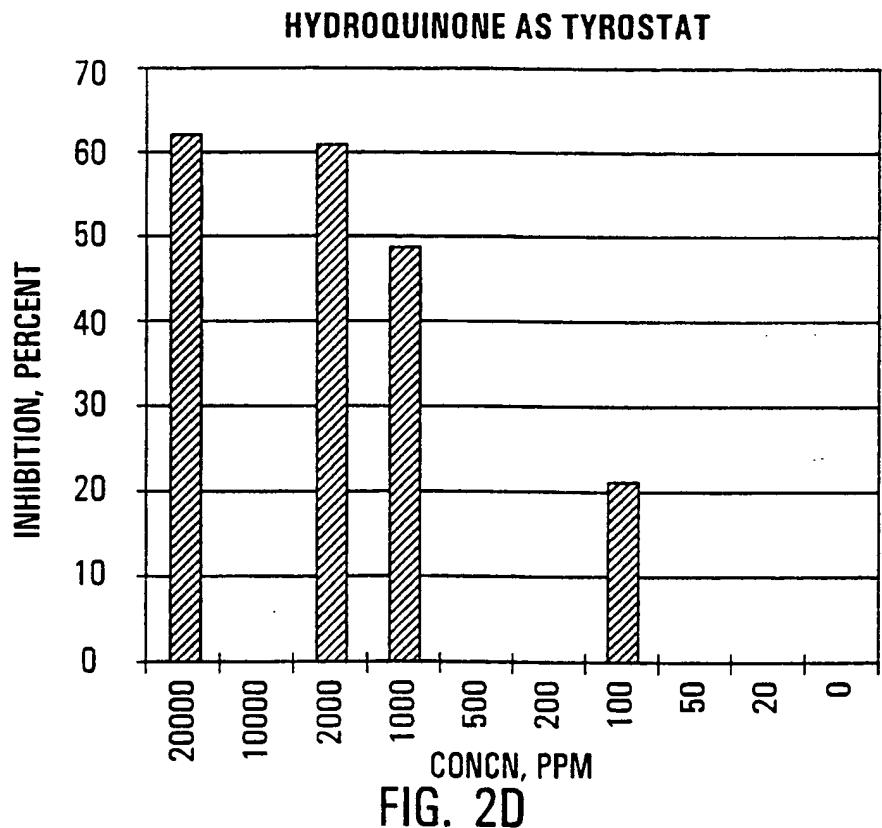
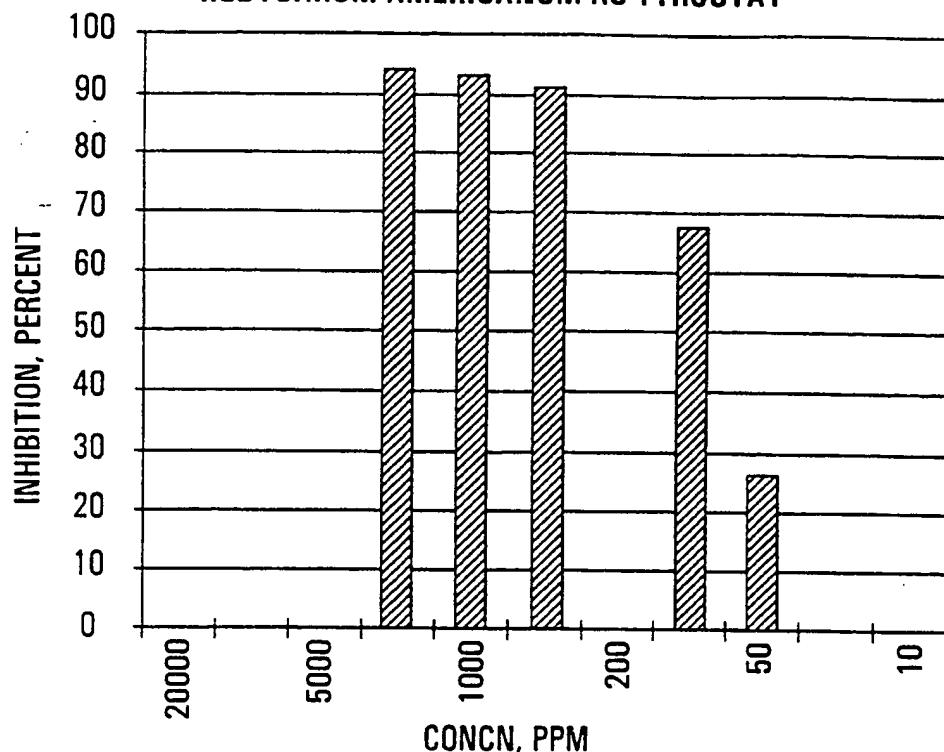
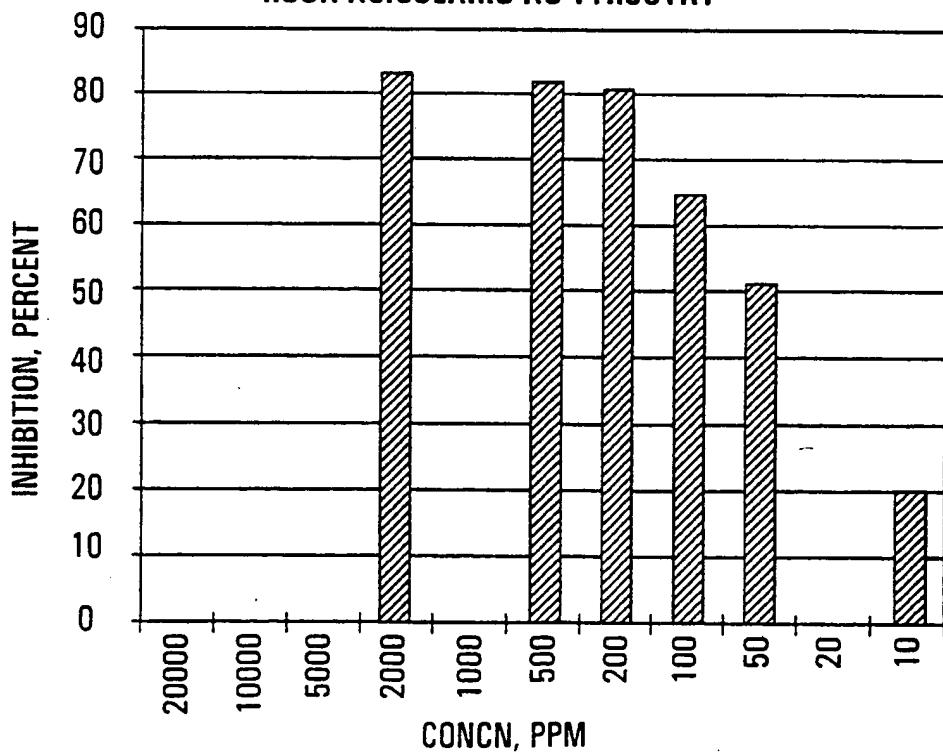


FIG. 2D

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**HEDYSARUM AMERICANUM AS TYROSTAT****FIG. 3A****ROSA ACICULARIS AS TYROSTAT****FIG. 3B**

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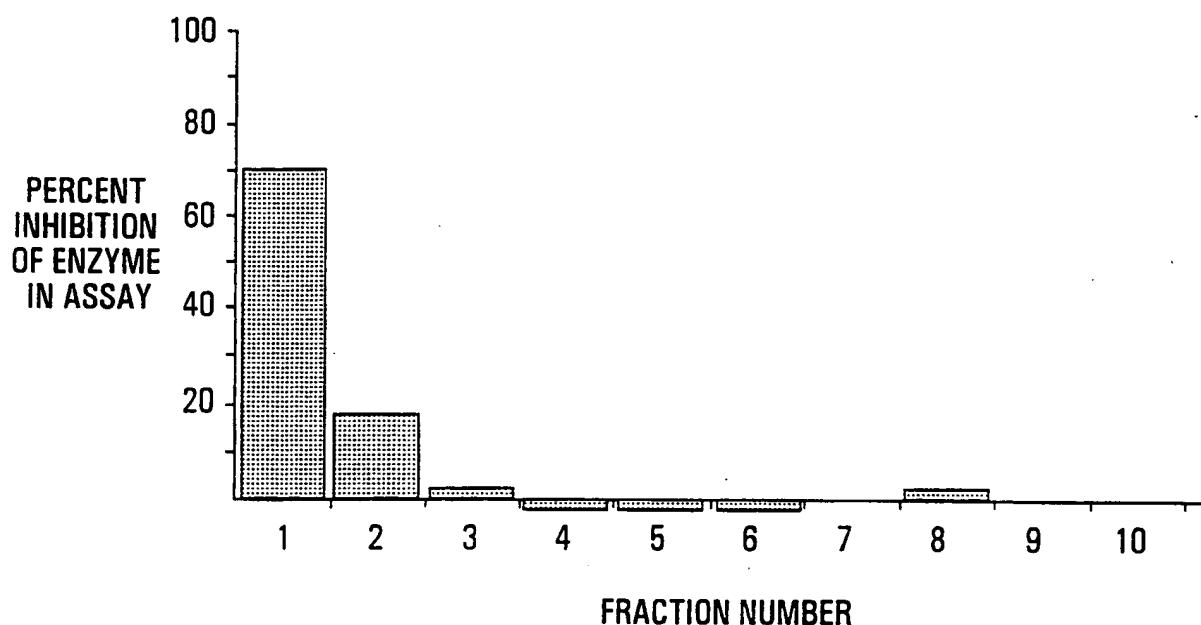


FIG. 4

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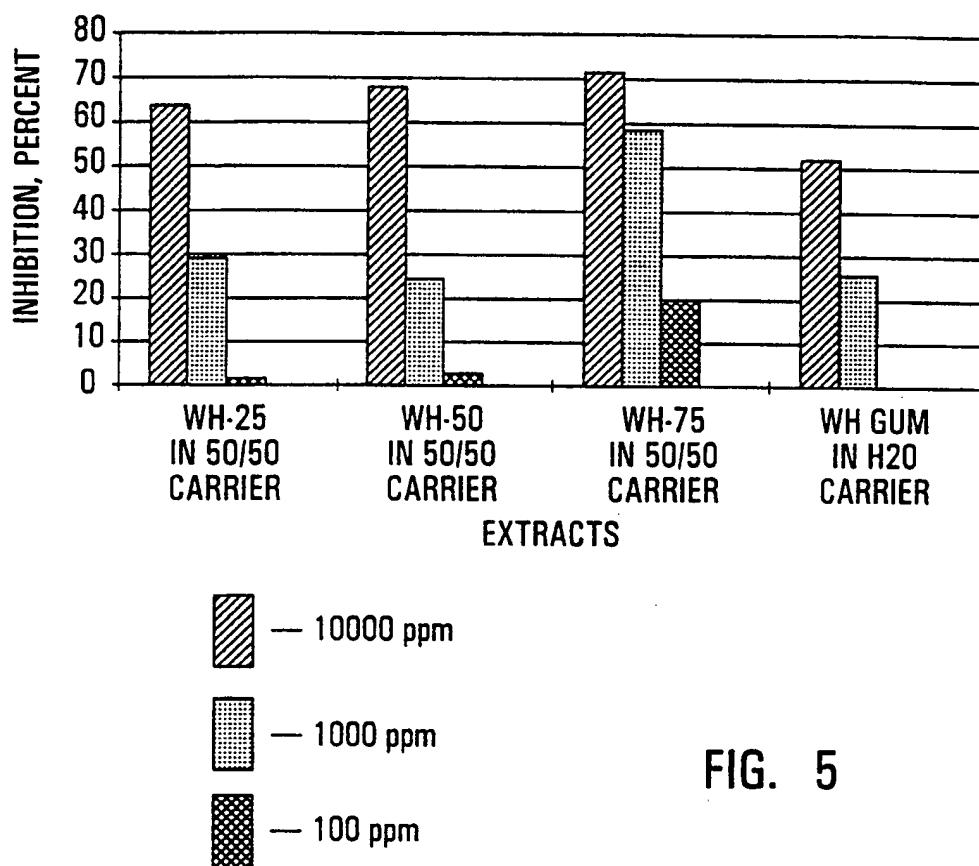


FIG. 5

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 99/00530

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 6 A61K35/78 A61K7/48

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PATENT ABSTRACTS OF JAPAN            vol. 017, no. 119 (C-1034),            12 March 1993 (1993-03-12)            &amp; JP 04 300812 A (MARUZEN SEIYAKU KK),            23 October 1992 (1992-10-23)            abstract</p> <p>---</p> <p>PATENT ABSTRACTS OF JAPAN            vol. 007, no. 003 (C-143),            7 January 1983 (1983-01-07)            &amp; JP 57 163307 A (POLA KASEI KOGYO KK),            7 October 1982 (1982-10-07)            abstract</p> <p>---</p> <p>---</p>	1-7, 10, 11, 19
X		1-7, 10, 11, 19

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

Date of mailing of the international search report

14 September 1999

28/09/1999

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## INTERNATIONAL SEARCH REPORT

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	PATENT ABSTRACTS OF JAPAN vol. 096, no. 005, 31 May 1996 (1996-05-31) & JP 08 012565 A (SHISEIDO CO LTD), 16 January 1996 (1996-01-16) abstract ---	1-7, 10, 11, 19
X	PATENT ABSTRACTS OF JAPAN vol. 011, no. 206 (C-433), 3 July 1987 (1987-07-03) & JP 62 029528 A (HIGASHIMARU SHOYU KK), 7 February 1987 (1987-02-07) abstract ---	1-5, 12, 19
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X	PATENT ABSTRACTS OF JAPAN vol. 096, no. 007, 31 July 1996 (1996-07-31) & JP 08 067615 A (NANBA TSUNEO; MIKIMOTO PHARMACEUT CO LTD), 12 March 1996 (1996-03-12) abstract ---	1-8, 10, 11, 19
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X	K.T. LEE ET AL.: "BIOLOGICAL SCREENING OF 100 PLANT EXTRACTS FOR COSMETIC USE (I): INHIBITORY ACTIVITIES OF TYROSINASE AND DOPA AUTO-OXIDATION." INTERNATIONAL JOURNAL OF COSMETIC SCIENCE, vol. 19, 1997, pages 291-298, XP002115303 LONDON, GB the whole document ---	1-7, 10, 13-17, 19
		-/-

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 99/00530

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOICHI IIDA ET AL.: "POTENT INHIBITORS OF TYROSINASE ACTIVITY AND MELANIN BIOSYNTHESIS FROM RHEUM OFFICINALE." PLANTA MEDICA, vol. 61, 1995, pages 425-428, XP002115304 STUTTGART, DE the whole document -----	1-7, 10, 11, 13-17, 19

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